The Chemical Action on Glucose of a Variety of Bacillus coli communis (*Escherich*), *obtained by Cultivation in Presence of a Chloroacetate.* (*Preliminary Notice.*)

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Penfold has shown (1) that when *Bacillus coli communis* (Escherich) is plated out on nutrient agar containing 0·5 per cent. sodium chloroacetate, a large number of small colonies and a few large ones are produced. The cultures obtained from some, but not all, of the large colonies were found to have lost the power of producing gas from glucose when grown in glucose peptone water in a test tube provided with a Durham gas tube. Under the same conditions these same organisms still produced gas from mannitol, but in diminished proportion. Several other intestinal organisms have since been found by Harden to behave in a similar manner on chloroacetate agar, whereas *B. lactis aërogenes*, which produces a different type of decomposition of sugar, has hitherto proved resistant to this method of selection. In order to ascertain what other modification the chemical action of the original strain had undergone, comparative quantitative experiments were carried out with the original and the selected organisms.

Product.	Percentage of sugar used.		Carbon atoms per molecule of glucose.		Harden's theory for *B. coli communis*. Carbon atoms.
	Normal *B. coli.*	Selected *B. coli.*	Normal *B. coli.*	Selected *B. coli.*	
Alcohol	17·22	5·5	1·35	0·43	1
Acetic acid	20·60	10·02	1·24	0·60	1
Formic acid	2·55	3·53	0·10 } 0·81	0·14 } 0·22	1
Carbon dioxide	17·30	2·00	0·71	0·08	
Lactic acid	40·60	77·6	2·43	4·66	3
Succinic acid	4·80	None	0·29	None	
	93·07	98·65	6·12	5·91	6
H (as gas) c.c. per grm. of sugar	80·6	12·6			
H as gas + H equivalent of formic acid	92·9	29·8			

The medium employed in both cases contained 10 grm. peptone and 20 grm. glucose per litre, 10 grm. of chalk being added per litre. The flasks were connected with the gas-collecting apparatus described by Harden, Thompson, and Young (2), and the air of the flask replaced by nitrogen before incubation. The estimations were made as previously described (3). The results of two such experiments are given in the table on p. 415, the products being expressed both in percentage of the sugar used and as the number of carbon atoms of the glucose molecule to which they correspond.

These results must be regarded as preliminary, but are of sufficient interest to justify some remark at the present stage of the work.

In the first place, it is to be noted that, although the selected organism gave no gas at all when tested by the Durham tube method, it yielded about 12·6 c.c. of hydrogen per gramme of sugar when grown anaërobically in presence of chalk as against 80·6 given by the original organism. Similarly the amount of gaseous CO_2 formed from the sugar was found to be 10·3 c.c. per gramme, but as this number is the difference of two large volumes (the total gaseous CO_2 + CO_2 dissolved in the medium — CO_2 liberated by the acids formed) no great accuracy attaches to it. The reason of this different behaviour is not understood and is at present being investigated.

Apart from this, an examination of the results shows that the main difference between the actions of the two bacteria is to be found in the increased proportion of lactic acid, and the correspondingly diminished proportion of the other products, formed by the selected organism.

Harden (3) has previously shown that the action of organisms closely allied to *B. coli communis* is roughly represented by the equation

$$(1)\quad 2C_6H_{12}O_6 + H_2O = 2C_3H_6O_3 + C_2H_6O + C_2H_4O_2 + 2CO_2 + 2H_2,$$

which requires the proportions of products shown in the last column of the table. Actually there is always less CO_2 than this produced, and with the strain of normal *B. coli communis* here employed there is also somewhat more alcohol and acetic acid and less lactic acid formed. The relations between the products of the selected organism appear, however, to be quite incompatible with this equation.

The explanation which suggests itself is that the products observed as the result of action of the normal organism are formed by three independent enzymes. One of these converts sugar into lactic acid:

$$(2)\quad C_6H_{12}O_6 = 2C_3H_6O_3.$$

The other probably produces alcohol, acetic acid, and formic acid, and the last of these is in all probability decomposed by a third enzyme into carbon dioxide and hydrogen:

$$(3) \quad C_6H_{12}O_6 + H_2O = C_2H_4O_2 + C_2H_6O + 2HCO_2H$$
$$= C_2H_4O_2 + C_2H_6O + 2CO_2 + 2H_2.$$

These enzymes may occur in different proportions in different individual organisms, and the process of selection would then result in the survival of an organism containing a large proportion of the lactic acid enzyme and a small proportion of that producing alcohol, acetic acid, and formic acid. It may be noted that the selected organism still retains the power of decomposing formic acid into CO_2 and H_2, and, moreover, its products do not contain more of this acid than those of the normal bacillus. The absence of gas formation, therefore, is not due to an accumulation of formic acid. It is thus seen that the change produced in the organism does not really cause its chemical action to approximate to that of *B. typhosus*, an organism which also yields no gas, but differs from *B. coli* by producing a large amount of formic acid.

The foregoing numbers are not sufficiently accurate to decide with certainty whether the relative proportions of alcohol, acetic acid, and formic acid produced by the selected organism are the same as those formed by the original bacillus. Taking the alcohol as standard, the carbon as formic acid (including gaseous CO_2) should be 0·27, instead of 0·22 observed, and that as acetic acid 0·41 instead of 0·6. The deviation in the last case may be due to the difficulty of effecting the quantitative separation of acetic acid from a large proportion of lactic acid.

Further experiments may be expected to throw light on these points.

If, however, the foregoing explanation be found correct, the study of the chemical action of these selected organisms should form a valuable means of investigation into the nature of the various processes of fermentation effected by bacteria, and the subject is being prosecuted from this point of view.

REFERENCES.

(1) Penfold, 'Roy. Soc. Med. Proc.,' 1911, p. 97.
(2) 'Biochemical Journal,' 1910, vol. 5, p. 230.
(3) Harden, 'Chem. Soc. Journ.,' 1901, p. 610.